

COMPOSITIONS AND METHODS FOR TREATING SLC26A4-ASSOCIATED HEARING LOSS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 63/024,466, filed May 13, 2020, the contents of which is hereby incorporated in its entirety.

BACKGROUND

[1] Hearing loss can be conductive (arising from the ear canal or middle ear), sensorineural (arising from the inner ear or auditory nerve), or mixed. Most forms of nonsyndromic deafness are associated with permanent hearing loss caused by damage to structures in the inner ear (sensorineural deafness), although some forms may involve changes in the middle ear (conductive hearing loss). The great majority of human sensorineural hearing loss is caused by abnormalities in the hair cells of the organ of Corti in the cochlea (poor hair cell function). The hair cells may be abnormal at birth, or may be damaged during the lifetime of an individual (e.g., as a result of noise trauma or infection).

SUMMARY

[2] The present disclosure provides the recognition that diseases or conditions associated with hearing loss can be treated via, e.g., the replacement or addition of certain gene products. The present disclosure further provides that gene products involved in the development, function, and/or maintenance of inner ear cells can be useful for treatment of diseases or conditions associated with hair cell and/or supporting cell loss. The present disclosure thus provides for the administration of compositions that result in expression of gene products involved in the development, function, and/or maintenance of inner ear cells including supporting cells and hair cells, and/or the use of such compositions in the treatment of hearing loss, or diseases or conditions associated with hearing loss. In one aspect, the present disclosure provides an AAV particle comprising a construct, wherein the construct comprising a coding sequence is operably linked to a promoter, wherein the coding sequence encodes an SLC26A4 gene. In another aspect, the present disclosure provides a composition comprising the AAV particle as disclosed herein. In another aspect, the present disclosure provides a composition comprising the AAV particle as disclosed herein for use in therapy, wherein the composition is to be introduced into the inner ear of a subject. In another aspect, the present disclosure provides the use of a composition comprising the AAV particle as disclosed herein in the manufacture of a medicament for treating hearing loss in a subject suffering from or at risk of hearing loss. In another aspect, the present disclosure provides a kit comprising a composition that comprises an AAV particle as disclosed herein, or a composition as disclosed herein. In some examples, a gene product can be encoded by an *SLC26A4* gene or a characteristic portion thereof. In some examples, a gene product can be pendrin protein or a characteristic portion thereof.

[3] The present disclosure further provides that AAV particles can be useful for administration of compositions that result in expression of gene products involved in the development, function, and/or maintenance of inner ear cells, and/or the treatment of hearing

loss, or diseases or conditions associated with hearing loss. As described herein, AAV particles comprise (i) a AAV polynucleotide construct (e.g., a recombinant AAV polynucleotide construct), and (ii) a capsid comprising capsid proteins. In some examples, an AAV polynucleotide construct comprises an *SLC26A4* gene or a characteristic portion thereof.

[4] The present disclosure further provides compositions comprising polynucleotide constructs comprising an *SLC26A4* gene or a characteristic portion thereof. In some examples, a construct may further include regulatory elements operably attached to a coding sequence. In certain examples, included regulatory elements facilitate tissue specific expression at physiologically suitable levels.

[5] The present disclosure also provides a genetically modified mouse whose genome comprises a modified *Slc26a4* gene. In some examples, the genetically modified mouse comprises a modified *Slc26a4* gene that encodes a polypeptide according to SEQ ID NO: 57. In some examples, the genetically modified mouse is of a mouse strain suitable for use in audiological analysis experiments. In some examples, the genetically modified mouse is of a mouse strain suitable for use in coordination analysis experiments. In some examples, the genetically modified mouse is not of CBA/CaJ or CBA/J strain. In some examples, the genetically modified mouse suitable for use in audiological analysis experiments is of FVB strain. In some examples, the genetically modified mouse suitable for use in audiological analysis experiments is of FVB, 129/Sv-+p+Tyr-c+Mgf-SIJ/J, A/HeJ, AKR/J, BALB/cByJ, BALB/cJ, BDP/J, BXSJ/MpJ, C3H/HeJ, C3H/HeOuJ, C3HeB/FeJ, C57BL/10J, C57BL/10SnJ, C57BL/6ByJ, CASA/RK, CAST/Ei, CBA/J, CZECH II/Ei, DBA/2HaSnm, FVB/NJ, HRS/J hrl+, MOLD/Rk, MOLF/Ei, MOLG/Dn, NON/LtJ, NZB/B1NJ, NZO/NiJ, NZW/LacJ, PERA/camEi, PERC/Ei, PL/J, RBA/Dn, RBF/DnJ, RF/J, RHJ/Le hrrh-J/+, RIIS/J, SEC/1ReJ, SENCARC/PtJ, SF/CamEi, SHR/GnEi, SJL/J, SM/J, SPRET/Ei, ST/bJ, or SWR/J strain. In some examples, a genetically modified mouse is treated with AAV particles, constructs, or compositions described herein.

[6] Also provided herein are methods of administering constructs and compositions described herein. In certain examples, administration involves surgical intervention and the delivery of rAAV particles comprising therapeutic constructs. In certain examples, AAV particles may be delivered to the inner ear of a subject in need thereof by surgical introduction through the round window membrane. In some examples, a purpose of an intervention is to

treat hearing loss in a subject. In some examples, efficacy of an intervention is determined through established tests, and measurements are compared to known control measurements.

DEFINITIONS

[7] The scope of the present disclosure is defined by the claims appended hereto and is not limited by certain embodiments described herein. Those skilled in the art, reading the present specification, will be aware of various modifications that may be equivalent to such described embodiments, or otherwise within the scope of the claims. In general, terms used herein are in accordance with their understood meaning in the art, unless clearly indicated otherwise. Explicit definitions of certain terms are provided below; meanings of these and other terms in particular instances throughout this specification will be clear to those skilled in the art from context.

[8] Use of ordinal terms such as “first,” “second,” “third,” etc., in the claims to modify a claim element does not by itself connote any priority, precedence, or order of one claim element over another or the temporal order in which acts of a method are performed, but are used merely as labels to distinguish one claim element having a certain name from another element having a same name (but for use of the ordinal term) to distinguish the claim elements.

[9] The articles “a” and “an,” as used herein, should be understood to include the plural referents unless clearly indicated to the contrary. Claims or descriptions that include “or” between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. In some embodiments, exactly one member of a group is present in, employed in, or otherwise relevant to a given product or process. In some embodiments, more than one, or all group members are present in, employed in, or otherwise relevant to a given product or process. It is to be understood that the present disclosure encompasses all variations, combinations, and permutations in which one or more limitations, elements, clauses, descriptive terms, etc., from one or more of the listed claims is introduced into another claim dependent on the same base claim (or, as relevant, any other claim) unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise. Where elements are presented as lists (e.g., in Markush group or similar format), it is to be understood

WHAT IS CLAIMED IS:

1. An AAV particle comprising a construct, wherein the construct comprising a coding sequence is operably linked to a promoter, wherein the coding sequence encodes an SLC26A4 gene.
2. The AAV particle of claim 1, wherein the SLC26A4 gene is a primate SLC26A4 gene.
3. The AAV particle of claim 1 or 2, wherein the SLC26A4 gene is a human SLC26A4 gene.
4. The AAV particle of any one of claims 1-3, wherein the promoter is an inducible promoter, a constitutive promoter, or a tissue-specific promoter.
5. The AAV particle of any one of claims 1-4, wherein the promoter is an inner ear cell-specific promoter.
6. The AAV particle of claim 5, wherein the inner ear cell-specific promoter is a GJB2 promoter, a GJB6 promoter, a SLC26A4 promoter, aTECTA promoter, a DFNA5 promoter, a COCH promoter, a NDP promoter, a SYN1 promoter, a GFAP promoter, a PLP promoter, a TAK1 promoter, a SOX21 promoter, a SOX2 promoter, a FGFR3 promoter, a PROX1 promoter, a GLAST1 promoter, a LGR5 promoter, a HES1 promoter, a HES5 promoter, a NOTCH1 promoter, a JAG1 promoter, a CDKN1A promoter, a CDKN1B promoter, a SOX10 promoter, a P75 promoter, a CD44 promoter, a HEY2 promoter, a LFNG promoter, or a S100b promoter.
7. The AAV particle of any one of claims 1-4, wherein the promoter is a CAG promoter, a CBA promoter, a CMV promoter, or a CB7 promoter.
8. The AAV particle of any one of claims 1-7, further comprising two AAV inverted terminal repeats (ITRs), wherein the two AAV ITRs flank the coding sequence and promoter.

9. The AAV particle of claim 8, wherein the two AAV ITRs are or are derived from AAV2 ITRs.
10. The AAV particle of any one of claims 1-9, further comprising an AAV capsid, wherein the AAV capsid is or is derived from an AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV-rh8, AAV-rh10, AAV-rh39, AAV-rh43 or AAV Anc80 capsid.
11. The AAV particle of claim 10, wherein the AAV capsid is an AAV Anc80 capsid.
12. A composition comprising the AAV particle of any one of claims 1-11.
13. The composition of claim 12, further comprising a pharmaceutically acceptable carrier.
14. A composition comprising the AAV particle of any one of claims 1-11 for use in therapy, wherein the composition is to be introduced into the inner ear of a subject.
15. The composition for use of claim 14, wherein the composition is to be introduced into the cochlea of the subject.
16. The composition for use of claim 15, wherein the composition is to be introduced via a round window membrane injection.
17. Use of a composition comprising the AAV particle of any one of claims 1-11 in the manufacture of a medicament for treating hearing loss in a subject suffering from or at risk of hearing loss.
18. The use of claim 17, wherein the composition is to be introduced into the inner ear of a subject.
19. The use of claim 18, wherein the composition is to be introduced into the cochlea of the subject.

20. The use of claim 19, wherein the composition is to be introduced via a round window membrane injection.
21. The use of claim 17, wherein the hearing loss is associated with a mutation in a SLC26A4 gene.
22. The use of claim 17, wherein the hearing loss and treatment of hearing loss are characterized as a function of ABR and/or Distortion Product Otoacoustic Emissions (DPOAE) measurements recorded prior to receiving any treatment and compared to ABR and/or DPOAE measurements after treatment.
23. A kit comprising a composition that comprises an AAV particle of any one of claims 1-11, or a composition of any one of claims 12-13.